ORIGINAL RESEARCH

Canagliflozin Increases Postprandial Total Glucagon-Like Peptide 1 Levels in the Absence of α-Glucosidase Inhibitor Therapy in Patients with Type 2 Diabetes: A Single-Arm, Non-randomized, Open-Label Study

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ABSTRACT

Introduction: To investigate canagliflozin-induced changes in postprandial total glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) levels in patients with type 2 diabetes mellitus (T2DM).
Methods: Forty-five patients with T2DM who had inadequate glycemic control (glycated hemoglobin ≥ 6.5%) with diet and exercise alone ($n = 15$, drug naïve) and in combination with either a stable dose of the α-glucosidase inhibitor acarbose ($n = 15$) or metformin ($n = 15$) received canagliflozin, a sodium-glucose cotransporter 2 inhibitor, at 100 mg once daily for 12 weeks. The primary endpoint was the change from baseline to week 12 in postprandial glucose and plasma levels of total GLP-1 and GIP during a meal tolerance test (MTT).

Results: Canagliflozin significantly reduced postprandial blood glucose (mean difference − 40.2 mg/mL at 60 min) and increased postprandial total GLP-1 (mean difference 1.8 pg/mL at 60 min) during an MTT. A transient reduction in the postprandial GIP level at only 30 min (mean difference − 80.3 pg/mL) during an MTT was observed. No changes in postprandial GLP-1 or GIP levels were seen after canagliflozin treatment as an add-on to acarbose in patients with T2DM. Acarbose treatment significantly decreased postprandial total GIP levels ($P < 0.05$) and tended to increase postprandial total GLP-1 levels ($P = 0.07$) compared to the other two treatments prior to canagliflozin.
Conclusion: Canagliflozin 100 mg increased postprandial total GLP-1 levels in the absence of acarbose, suggesting that it may upregulate GLP-1 secretion through delayed glucose absorption in the upper intestine, as with the α-glucosidase inhibitor.
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INTRODUCTION

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are a new class of diabetic medications that inhibit renal glucose reabsorption, promote glucosuria, and effectively reduce glycated hemoglobin (HbA1c), providing hypoglycemia-free glucose control in patients with type 2 diabetes mellitus (T2DM) [1, 2]. Currently, of the six SGLT2 inhibitors commercially available in Japan, canagliflozin has a lower SGLT2 selectivity of 155-fold over SGLT1 and 250–3000-fold selectivity compared with other SGLT2 inhibitors [3].

SGLT1 is highly expressed on the brush-border membrane of enterocytes in the proximal intestine and plays a role in glucose absorption [4, 5]. Thus, the intestinal SGLT1 inhibition lowers serum glucose levels and conversely augments distal intestinal glucose levels. Incretins, such as glucose-dependent insulinotropic polypeptide (GIP) from K cells and glucagon-like peptide 1 (GLP-1) from L cells in the intestine, are hormones that are released from the gut into the bloodstream in response to nutrient ingestion [6–8]. SGLT1 is also expressed in the proximal tubule, heart, skeletal muscle, and others [9, 10]. Experiments suggest that canagliflozin exhibited modest potency against SGLT1 in the intestine as a result of delayed glucose absorption and increased GLP-1 secretion [11], as well as SGLT2 inhibition in the proximal tubule. In clinical studies with healthy subjects [12, 13], a single dose of orally administered canagliflozin 300 mg transiently delayed intestinal glucose absorption, probably as a result of SGLT1 inhibition, which resulted in a reduction in plasma GIP levels and an increase in plasma GLP-1 levels [12].

The α-glucosidase inhibitors (α-GIs) inhibit α-glucosidase enzymes in the intestinal brush borders, leading to a delay in the digestion of complex carbohydrates and absorption of glucose in the upper part of the small intestine. This process is mediated by lower GIP levels and improves postprandial hyperglycemia [14–16]. Furthermore, a delay in glucose absorption by α-GIs accelerates GLP-1 secretion in the lower intestine and, consequently, increases plasma GLP-1 levels, which partly contributes to the inhibition of postprandial hyperglycemia [15, 16].

Metformin has also been reported to increase plasma GLP-1 levels after a test meal in patients with T2DM [17–19]. The mechanisms underlying the increased plasma GLP-1 levels associated with metformin treatment are poorly understood. Metformin has been suggested to inhibit the apical sodium-dependent bile acid transporter in the ileum, thereby increasing the luminal concentration of bile acids in the ileum and colon [18]. Exogenous bile acids increase circulating GLP-1 in clinical studies [20, 21]. Thus, metformin induces GLP-1 secretion indirectly through an increase in bile acids in the intestine.

The present study aimed to investigate the effects of canagliflozin 100 mg once daily for 12 weeks on blood glucose control and postprandial total GLP-1 levels in Japanese patients with T2DM, as either single or combination therapy with α-GI or metformin, both of which are known to potentiate GLP-1 secretion.

METHODS

Study Subjects

This was a 12-week, open-label study conducted at Naka Kinen Clinic (Ibaraki, Japan). The study protocol was approved by the Naka Kinen Clinic Institutional Review Board, Ibaraki, Japan, and is registered in the University Hospital Medical Information Network Clinical Trials Registry as UMIN000018345. All procedures performed in this study involving human participants were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1964, as revised in 2013. Informed consent was obtained from all individual participants included in the study. Participants were compensated for their time.

Eligible participants were patients aged at least 20 years and less than 75 years with T2DM who met the following criteria: (1) had a baseline HbA1c between 6.5% and 8.5%, and (2) underwent regular dietary or exercise therapy for at least 8 weeks, or took stable doses of oral glucose-lowering agents of α-GI or metformin

for at least 8 weeks. Patients who met at least one of the following criteria were excluded from the study: (1) had type 1 diabetes mellitus or secondary diabetes mellitus; (2) had a contraindication to canagliflozin; (3) had severe renal dysfunction (estimated glomerular filtration rate less than 30 mL/min/1.73 m^2); (4) had a body mass index (BMI) less than 22 kg/m^2; (5) were receiving glucose-lowering therapy by insulin, GLP-1 analogues, or other SGLT2 inhibitors; (6) were pregnant, breast-feeding or planning to be pregnant; or (7) were deemed by the investigator to be not suitable, e.g., patients with a complication or history of cerebral or myocardial infarction.

Study Design

Patients with T2DM inadequately controlled by a regular diet and exercise therapy regimen (classified as the drug-naïve group), acarbose (the α-GI group), or metformin (the metformin group) for at least 8 weeks were enrolled and given canagliflozin 100 mg once daily for 12 weeks. The meal tolerance tests (MTTs) were carried out before and after canagliflozin treatment for 12 weeks. A meal was about 504 kcal and consisted of 63.1% carbohydrate, 12.4% protein, and 23.2% fat. For the MTTs, canagliflozin 100 mg was administered 30 min before the start of the meal and blood samples were collected at four time points: before a meal and 30, 60, and 90 min after a meal to measure blood glucose, total GIP, total GLP-1, and immunoreactive insulin levels.

To assess glycemic control and anthropometric variables, HbA1c, glycoalbumin, body weight, and BMI were measured before and every 4 weeks during the canagliflozin treatment period of 12 weeks. The waist circumference was also measured before and after a 12-week treatment with canagliflozin.

Measurement of Total GIP and GLP-1 Levels, Immunoreactive Insulin, Homeostatic Model Assessment (HOMA)-R, and HOMA-Beta Cell Function (HOMA-B)

Plasma total GIP levels were measured with an ELISA kit that had 100% cross-reactivity to human active GIP and inactive GIP (EMD Millipore Corporation, MO, USA) [22, 23]. The detection limit of total GIP in this assay was 0.8 pmol/L. Plasma total GLP-1 levels were determined by an electrochemiluminescent immunoassay kit (Meso Scale Diagnostic LLC, MD, USA) [23]. The detection limit of total GLP-1 in this assay was 0.4 pmol/L. Values below the detection limit were calculated as half values of the detection limit. Serum immunoreactive insulin levels were measured with a chemiluminescent enzyme immunoassay kit from SRL Inc (Tokyo, Japan). Two samples were missing in both the drug-naïve group and the metformin group. HOMA-R, an index of insulin resistance, and HOMA-B were calculated as follows [24]; HOMA-R = insulin × fasting blood glucose/405, HOMA-B = insulin × 360/(fasting blood glucose − 63).

Statistical Analysis

Data in all tables and figures are presented as the arithmetic mean ± standard deviation (SD) unless otherwise stated. An incremental area under the curve (ΔAUC; defined as AUC above the value before a meal in an MTT) from 0 to 90 min was calculated using the trapezoidal rule for blood glucose, total GIP, and total GLP-1 levels during MTTs. For HbA1c, glycoalbumin, body weight, BMI, waist circumference, HOMA-R, and HOMA-B, statistical differences between pre- and post-treatment at each time point (4, 8, 12 weeks) were analyzed using paired t tests. The same method was used to detect the statistical significance for blood glucose, total GIP and GLP-1, and immunoreactive insulin levels during an MTT between pre- and post-treatment at each time point (before a meal, 30, 60, and 90 min after a meal). ΔAUCs were analyzed using paired t tests to detect the difference between pre- and post-treatment. Analyses were performed in all patients by group. In addition, a one-way analysis of variance was used to compare baseline values of ΔAUCs, the percentage change of ΔAUCs from baseline, and continuous data on patients' demographics/baseline characteristics between groups, followed by the Bonferroni post hoc test. $P < 0.05$ at a two-sided level was considered statistically significant. Pearson's

correlation coefficient tests were applied to assess the correlation between numerical variables. Statistical analyses were performed using SAS ver. 9.2 (SAS Institute Japan Ltd., Tokyo, Japan).

RESULTS

Patient Disposition and ΔAUCs at Baseline

Forty-eight patients with T2DM, who were eligible for the study and provided informed consent, were enrolled. Three patients were excluded from the study because of withdrawal of informed consent. The remaining 45 patients were enrolled and received canagliflozin 100 mg. Each group (drug naïve, α-GI, and metformin) consisted of 15 patients. All 45 participants completed the study over 12 weeks and underwent examinations specified in the study protocol. Dose levels of α-GI (acarbose) and metformin were 150–300 and 500–-2250 mg/day, respectively.

Patient demographics and baseline characteristics by group were similar between groups except for the sex ratio, age, fasting blood glucose, and HOMA-R (Table 1). The mean fasting blood glucose and HOMA-R were significantly higher in the α-GI group than that in the metformin group ($P < 0.01$) and the drug-naïve group ($P < 0.05$), respectively before treatment with canagliflozin (Table 1), but there were no statistically significant difference among the groups after treatment with canagliflozin for 12 weeks. Significantly lower baseline ΔAUC values for postprandial glucose ($P < 0.01$; Fig. 1e) and total GIP levels ($P < 0.05$; Fig. 3e) were noted in the α-GI group than in the other groups. ΔAUC of the total GLP-1 level was higher in the α-GI group than in the other groups, although it did not reach statistical significance ($P = 0.07$; Fig. 2e).

Blood Parameters After an MTT

The time courses of blood glucose (Fig. 1a–d), total GLP-1 (Fig. 2a–d), total GIP (Fig. 3a–d), and immunoreaction insulin (Fig. 4a–d) levels, together with their ΔAUCs, during the MTTs

before and after treatment with canagliflozin for 12 weeks for all patients and by group showed significant changes in all parameters analyzed.

Blood glucose levels were significantly reduced at all time points (mean difference $\pm$ SD -17.7 ± 21.6, -35.8 ± 28.5, -40.2 ± 28.7, and -37.4 ± 32.7 mg/dL at 0, 30, 60, and 90 min after a meal, respectively), regardless of the group ($P < 0.01$), and, as a result, ΔAUCs significantly decreased ($P < 0.01$). Despite lower baseline postprandial hyperglycemia in the α-GI group than in the other groups before treatment with canagliflozin, a lowering effect on postprandial glucose levels was observed in this group, which was similar in extent to that in the metformin group after treatment with canagliflozin for 12 weeks. The percentage change in ΔAUC of blood glucose levels from baseline in the α-GI group was small, but not significantly between the two groups (Fig. 1f).

Postprandial plasma total GLP-1 levels after treatment with canagliflozin were significantly increased at all time points ($P < 0.05$ or $P < 0.01$) in all patients (mean difference $\pm$ SD 0.1 ± 1.8, 1.1 ± 3.3, 1.8 ± 3.1, and 1.1 ± 3.2 pmol/L at 0, 30, 60, and 90 min after a meal, respectively) and in the metformin group (mean difference $\pm$ SD -0.6 ± 1.7, 2.7 ± 4.0, 2.3 ± 2.7, and 1.8 ± 3.1 pmol/L at 0, 30, 60, and 90 min after a meal, respectively), and at 60 min in the drug-naïve group (mean difference $\pm$ SD -0.5 ± 0.5, 0.9 ± 2.5, 2.0 ± 2.3, and 1.4 ± 2.6 pmol/L at 0, 30, 60, and 90 min after a meal, respectively) during the MTT ($P < 0.01$), which led to a significant increase in ΔAUCs ($P < 0.01$).

In contrast, the postprandial total GIP level was significantly reduced at only 30 min in all patients (mean difference $\pm$ SD 2.4 ± 9.9, -16.1 ± 19.0, -7.60 ± 28.0, and 2.36 ± 27.9 pmol/L at 0, 30, 60, and 90 min after a meal, respectively), the drug-naïve group (mean difference $\pm$ SD 0.2 ± 8.7, -24.0 ± 15.9, -12.3 ± 27.8, and -5.5 ± 21.2 pmol/L at 0, 30, 60, and 90 min after a meal, respectively), and the metformin group (mean difference $\pm$ SD 3.1 ± 11.1, -17.5 ± 21.2, -7.8 ± 33.7, and 5.1 ± 32.5 pmol/L at 0, 30, 60, and 90 min after a meal, respectively) during the MTT ($P < 0.01$).

Table 1 Baseline characteristics

	All subjects ($n = 45$ or 41^e)	Drug-naïve group ($n = 15$ or 13^e)	α-GI group ($n = 15$)	Metformin group ($n = 15$ or 13^e)	3-Group comparisons
Male, n (%)	30 (66.7%)	13 (86.7%)	11 (73.3%)	6 (40.0%)	$P = 0.04^a$
Age, years	54.0 ± 10.9	55.1 ± 10.0	48.3 ± 11.5	58.5 ± 9.1	$P = 0.03^b$
HbA1c, % (mmol/mol)	7.1 ± 0.6 (53.8 ± 7.0)	7.3 ± 0.7 (55.4 ± 7.8)	7.2 ± 0.8 (54.7 ± 8.5)	6.9 ± 0.3 (51.3 ± 3.2)	$P = 0.19$
Glycoalbumin, %	17.5 ± 2.3	18.3 ± 2.0	17.8 ± 2.5	16.5 ± 2.0	$P = 0.08$
Fasting blood glucose, mg/dL	140.0 ± 23.8	139.9 ± 20.3	152.8 ± 28.4	127.4 ± 14.4	$P = 0.01^c$
Body weight, kg	76.4 ± 15.9	75.3 ± 9.7	81.4 ± 19.6	72.4 ± 16.3	$P = 0.29$
Body mass index, kg/cm^2	28.3 ± 4.2	27.3 ± 3.4	29.2 ± 5.5	28.3 ± 3.4	$P = 0.48$
Waist circumference, cm	96.9 ± 10.6	93.9 ± 7.0	100.6 ± 13.2	96.1 ± 10.2	$P = 0.22$
HOMA-R	3.6 ± 2.2	2.8 ± 1.0	4.9 ± 3.0	3.0 ± 1.5	$P = 0.03^d$
HOMA-B	49.3 ± 24.1	41.2 ± 14.4	52.0 ± 24.8	54.3 ± 30.1	$P = 0.43$
Duration of type 2 diabetes, years	5.2 ± 3.7	3.8 ± 3.5	5.6 ± 3.7	6.2 ± 3.8	$P = 0.18$
Complication, n (%)					
Retinopathy	11 (24.4%)	4 (26.7%)	4 (26.7%)	3 (20.0%)	$P = 0.89$
Nephropathy	10 (22.2%)	5 (33.3%)	2 (13.3%)	3 (20.0%)	$P = 0.55$
Nerve disorders	8 (17.8%)	2 (13.3%)	3 (20.0%)	3 (20.0%)	$P = 1.00$

Data are presented as the mean ± SD

GI glucosidase inhibitor, *SD* standard deviation

[a] $P < 0.05$ for the comparison of male sex (% of total) between the drug-naïve group and the metformin group

[b] $P < 0.05$ for the comparison of age between the α-GI group and the metformin group

[c] $P < 0.05$ for the comparison of fasting blood glucose between the α-GI group and the metformin group

[d] $P < 0.05$ for the comparison of HOMA-R between the drug-naïve group and the α-GI group

[e] Two samples (HOMA-R and HOMA-B) were missing in both the drug-naïve group and the metformin group

ΔAUC significantly decreased in all patients and the drug-naïve group ($P < 0.05$), but not in the metformin group.

In the α-GI group, no changes in postprandial total GLP-1 (mean difference ± SD 0.9 ± 2.4, − 0.3 ± 2.6, 1.0 ± 4.0, and 0.3 ± 3.9 pmol/L at 0, 30, 60, and 90 min after a meal, respectively) or GIP levels (mean difference ± SD 4.0 ± 10.1, − 6.8 ± 16.6, − 2.8 ± 22.7, and 7.5 ± 29.0 pmol/L at 0, 30, 60, and 90 min after a meal, respectively) were seen during the MTT after treatment with

canagliflozin. The percentage changes in ΔAUC of total GLP-1 and GIP levels from baseline in the α-GI group were both slight, but the latter was not significantly different between the groups (Figs. 2f, 3f, respectively).

Immunoreactive insulin levels after treatment with canagliflozin were significantly reduced at all time points in all patients (mean difference ± SD − 1.5 ± 2.8, − 5.7 ± 6.9, − 7.1 ± 8.8, and − 6.9 ± 11.5 mg/dL at 0, 30, 60, and 90 min after a meal, respectively), at 30, 60, and 90 min in the drug-naïve group, at 30
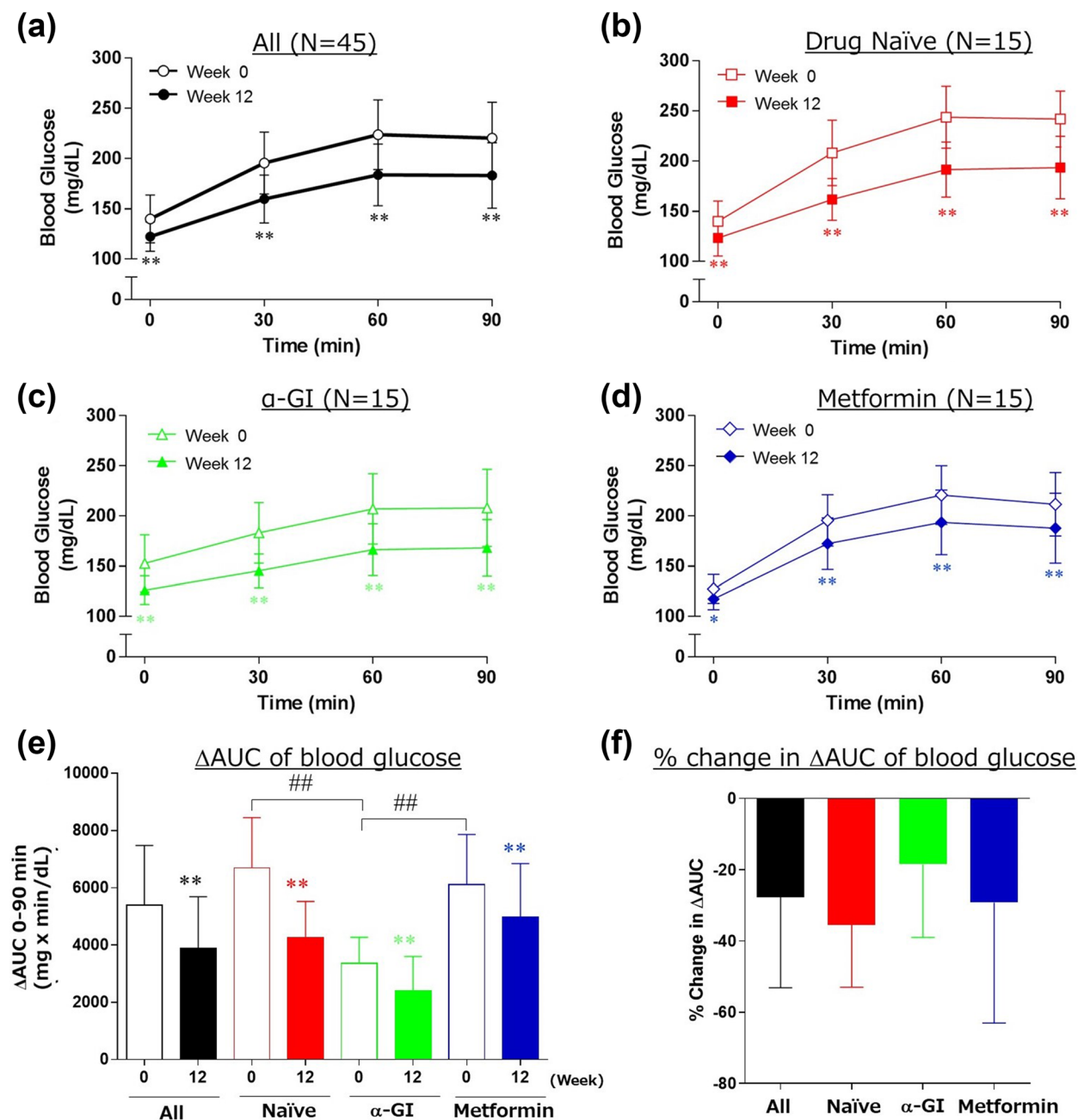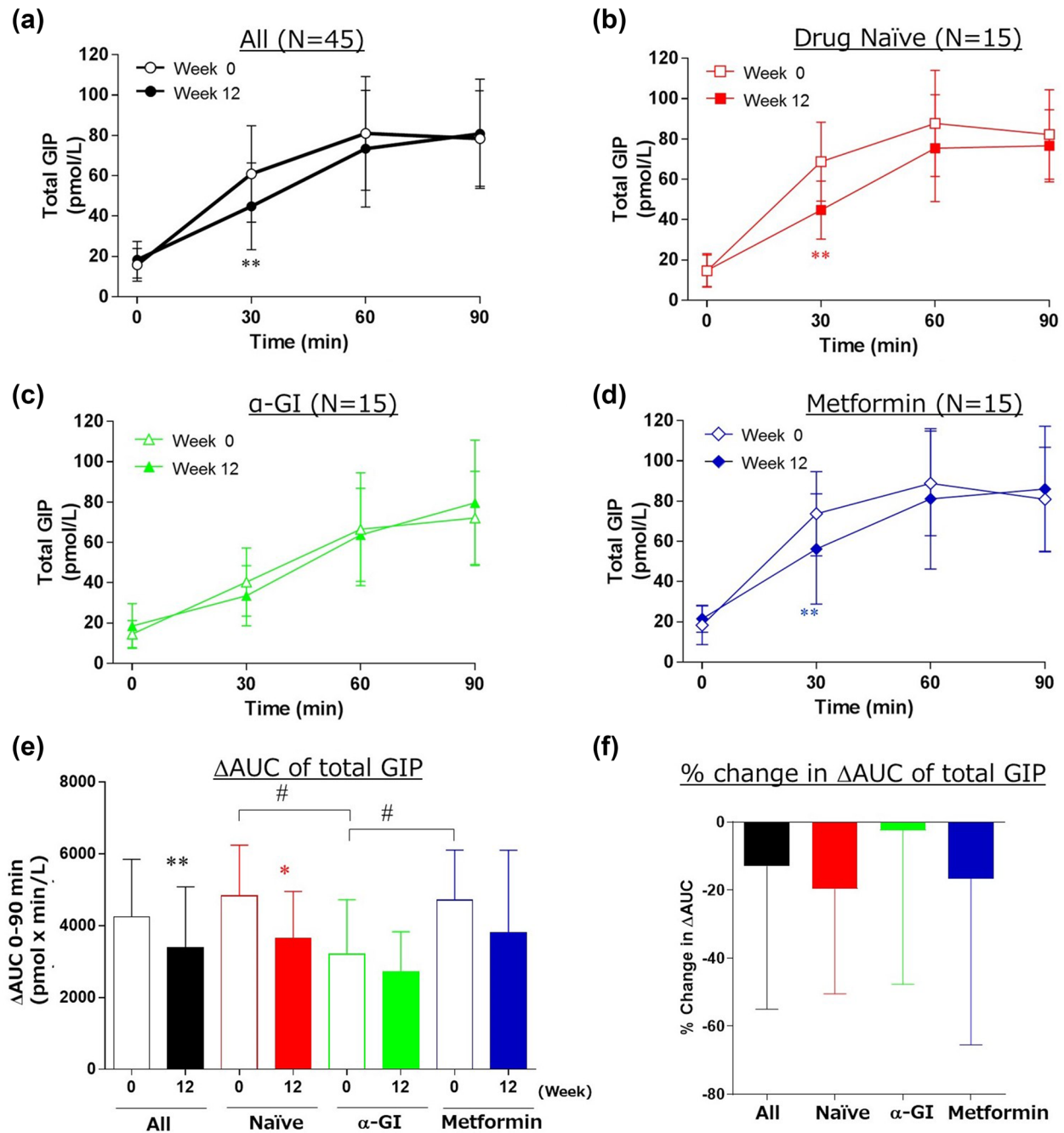
Fig. 1 Effect of canagliflozin on postprandial blood glucose in all patients (**a**), the drug-naïve group (**b**), the α-GI group (**c**), and the metformin group (**d**), and on an incremental area under the curve (ΔAUC) from 0 to 90 min (**e**), and the percentage change in ΔAUC (**f**). Data are presented as the mean ± SD. $^*P < 0.05$ and $^{**}P < 0.01$ in comparison with baseline (week 0) at each time point and that between pre- and post-treatment of canagliflozin in ΔAUC. $^{\#\#}P < 0.01$ for the comparison of ΔAUC at baseline between the α-GI group and the other two groups. No statistical differences were noted between the three groups in the percentage change in ΔAUC. GI glucosidase inhibitor, SD standard deviation
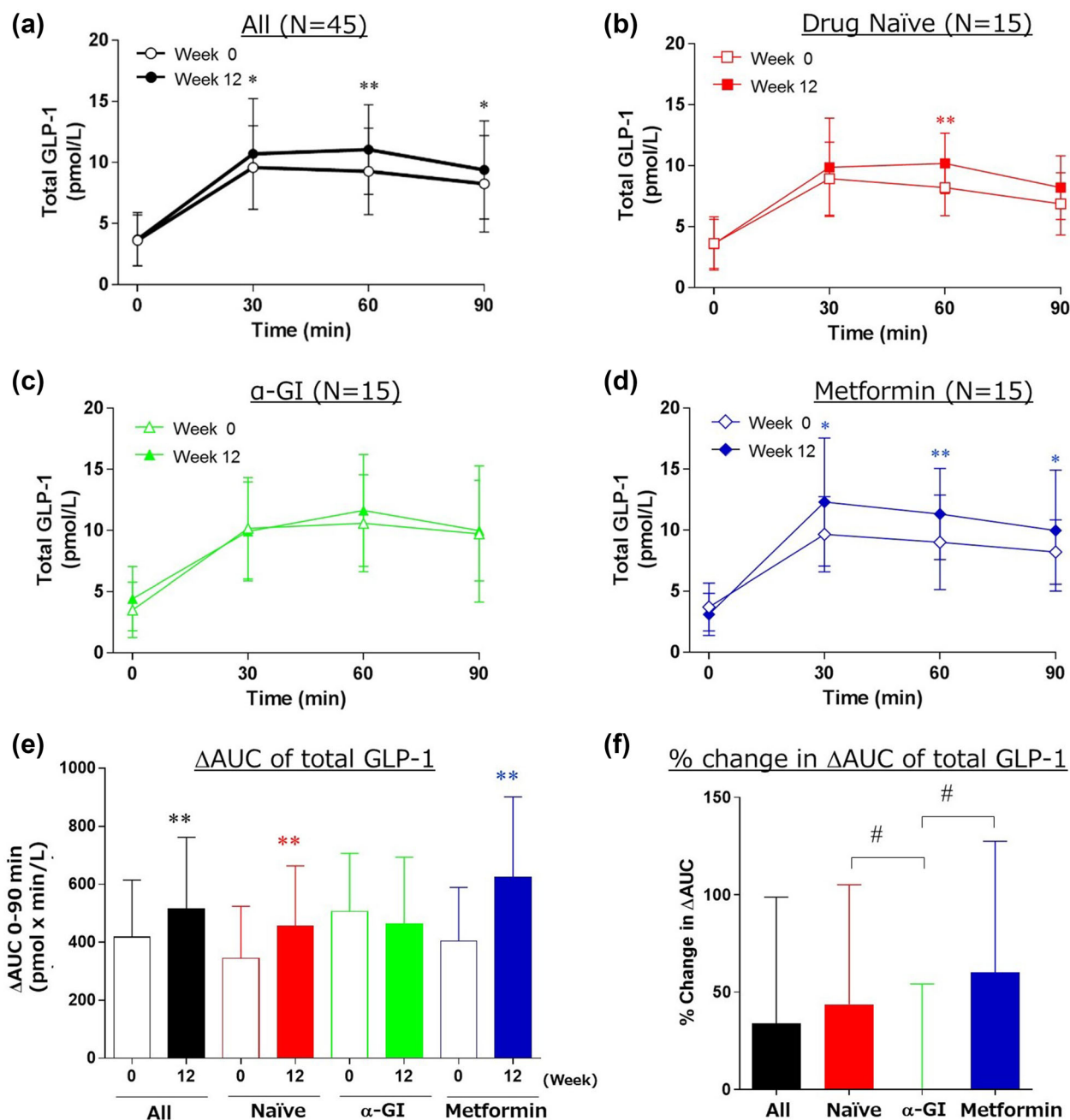
Fig. 2 Effect of canagliflozin on the postprandial plasma total GLP-1 level in all patients (**a**), the drug-naïve group (**b**), the α-GI group (**c**), and the metformin group (**d**), and on an incremental area under the curve (ΔAUC) from 0 to 90 min (**e**), and the percentage change in ΔAUC (**f**). Data are presented as the mean ± SD. *$P < 0.05$ and **$P < 0.01$ in comp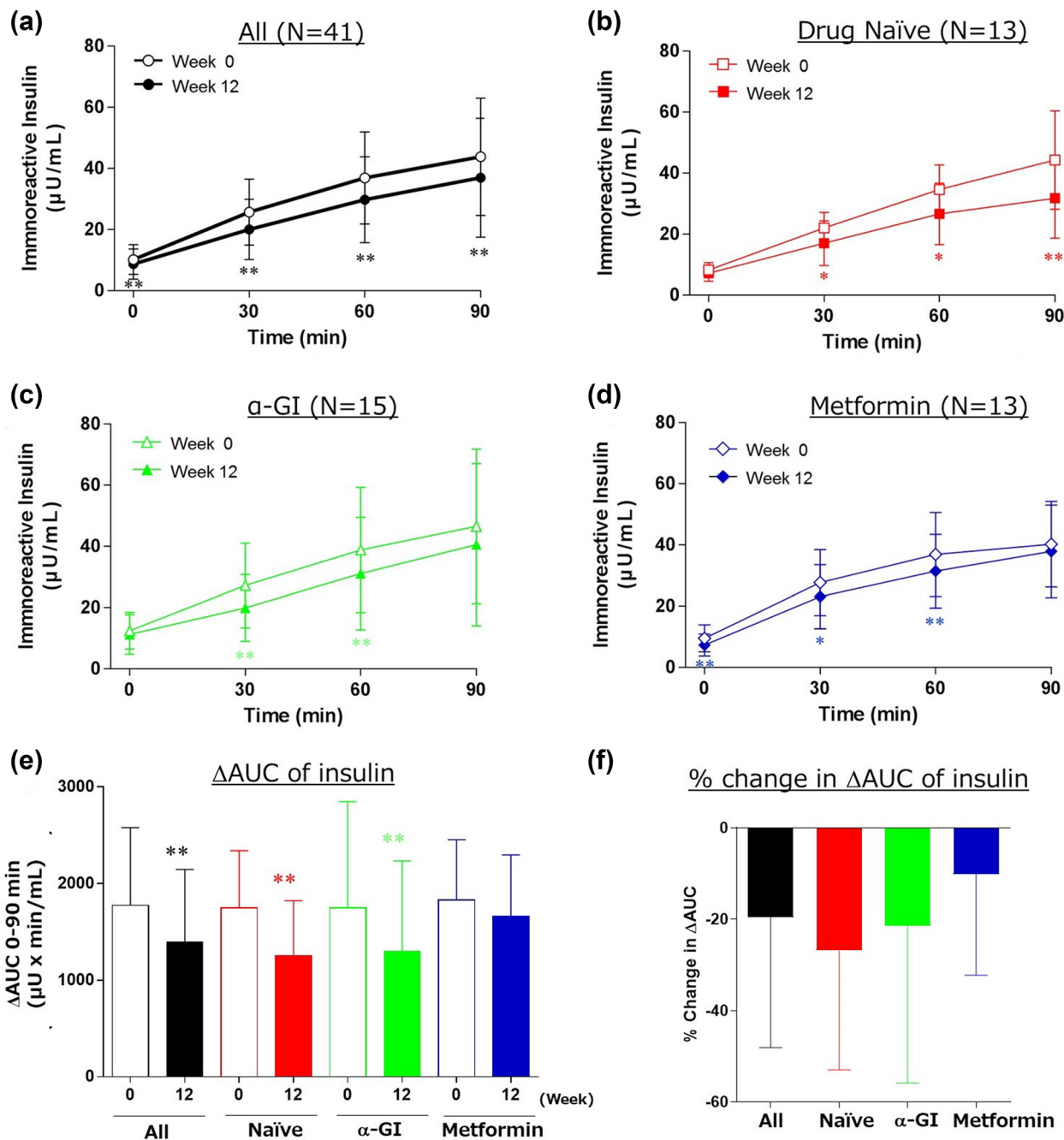arison with baseline (week 0) at each time point and that between pre- and post-treatment of canagliflozin in ΔAUC. No statistical differences were noted between the 3 groups in ΔAUC at baseline ($P = 0.07$). #$P < 0.05$ for the comparison of the percentage change in ΔAUC between the α-GI group and the other two groups. GLP-1 glucagon-like peptide 1, GI glucosidase inhibitor, SD standard deviation

Fig. 3 Effect of canagliflozin on the postprandial plasma total GIP level in all patients (a), the drug-naïve group (b), the α-GI group (c), and the metformin group (d), and on an incremental area under the curve (ΔAUC) from 0 to 90 min (e), and the percentage change in ΔAUC (f). Data are presented as the mean ± SD. *P < 0.05 and **P < 0.01 in comparison with baseline (week 0) at each time point and that between pre- and post-treatment of canagliflozin in ΔAUC. #P < 0.05 for the comparison of ΔAUC at baseline between the α-GI group and the other two groups. No statistical differences were noted between the three groups in the percentage change in ΔAUC. GIP glucose-dependent insulinotropic polypeptide, GI glucosidase inhibitor, SD standard deviation

Fig. 4 Effect of canagliflozin on the postprandial serum immunoreactive insulin level in all patients (**a**), the drug-naïve group (**b**), the α-GI group (**c**), and the metformin group (**d**), and on an incremental area under the curve (ΔAUC) from 0 to 90 min (**e**), and the percentage change in ΔAUC (**f**). Data are presented as the mean ± SD. *$P < 0.05$ and **$P < 0.01$ in comparison with baseline (week 0) at each time point and that between pre- and post-treatment of canagliflozin in ΔAUC. No statistical differences were noted between the three groups in ΔAUC at baseline and the percentage change in ΔAUC. GIP glucose-dependent insulinotropic polypeptide, GI glucosidase inhibitor, SD standard deviation
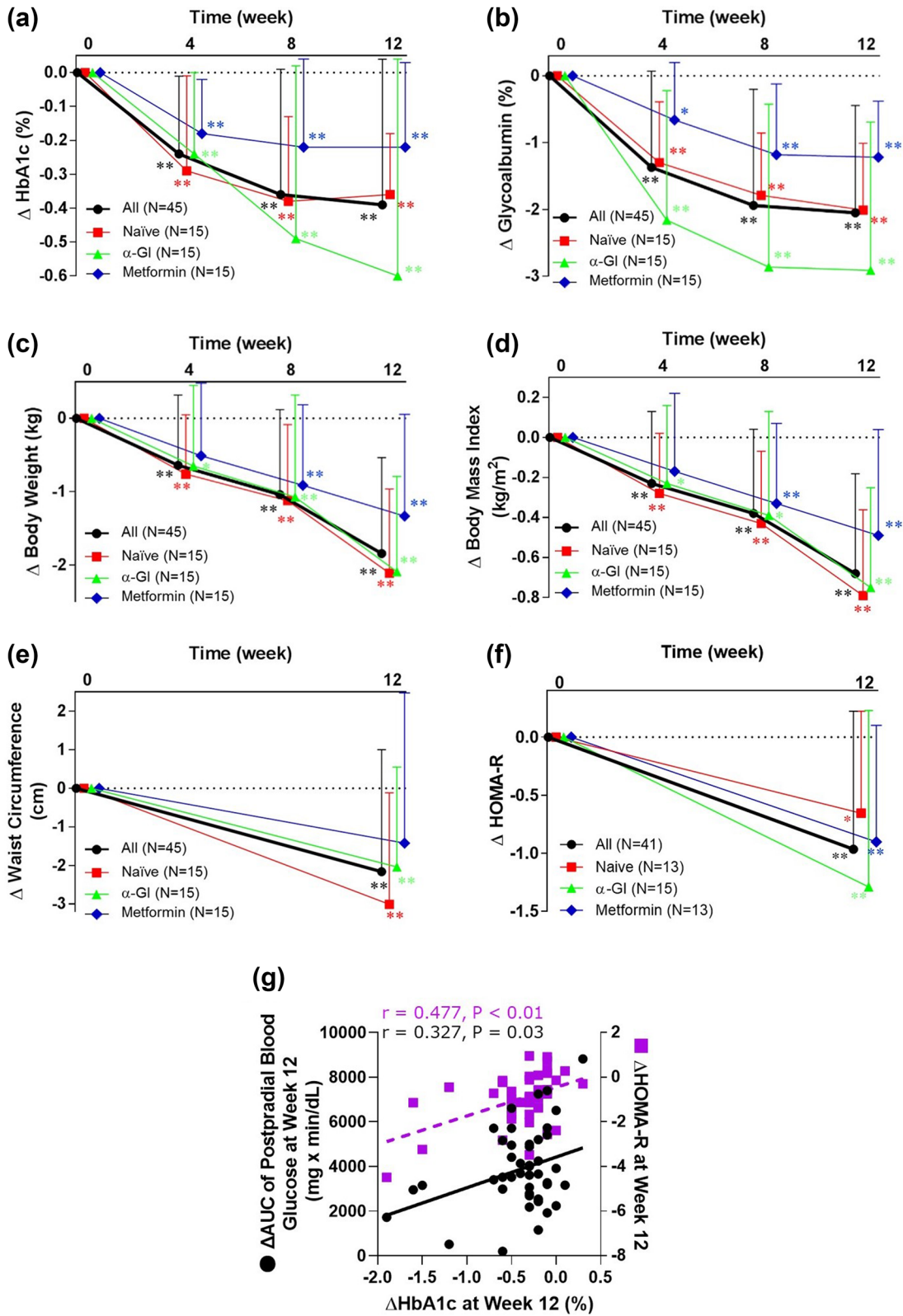
◀**Fig. 5** Changes in HbA1c (**a**), glycoalbumin (**b**), body weight (**c**), body mass index (**d**), waist circumference (**e**), and HOMA-R (**f**) during the treatment with canagliflozin, and relationship between the change in HbA1c and ΔAUC of postprandial blood glucose or the change in HOMA-R at week 12 (**g**). Data are presented as the mean ± SD. *$P < 0.05$ and **$P < 0.01$ in comparison with baseline (week 0). SD standard deviation, GI glucosidase inhibitor, *r* correlation coefficient

and 60 min in the α-GI group, and 0, 30, and 60 min in the metformin group ($P < 0.05$ or $P < 0.01$). ΔAUC significantly decreased in all patients, the drug-naïve group, and the α-GI group ($P < 0.01$), but not in the metformin group. The percentage change in ΔAUC of immunoreactive insulin levels from baseline was not significantly different between the groups (Fig. 4f).

To examine the relationship between blood glucose, total GLP-1, total GIP, and immunoreactive insulin levels, correlation analysis was performed. There was no correlation between each parameter value of the percentage change in ΔAUC (Fig. 1 in the supplementary material).

Clinical Parameters

HbA1c ($P < 0.05$) and glycoalbumin ($P < 0.01$) decreased significantly 4 weeks after the start of canagliflozin treatment and remained low up to 12 weeks in all patients and in each group. The changes in HbA1c (Fig. 5a) and glycoalbumin (Fig. 5b) levels from the baseline after treatment with canagliflozin for 12 weeks were greater in the α-GI group than in the other groups. Body weight (Fig. 5c) and BMI (Fig. 5d) showed a gradual reduction in all patients and in each group from week 4 to week 12 after treatment with canagliflozin. The waist circumference showed a significant reduction after treatment with canagliflozin in all patients, the drug-naïve and α-GI groups ($P < 0.01$), but not in the metformin group (Fig. 5e). HOMA-R showed a significant reduction after treatment with canagliflozin in all patients and in each group (Fig. 5f), but HOMA-B was not significantly changed after treatment with canagliflozin for

12 weeks in all patients (mean difference ± SD 4.6 ± 22.7) and in each group.

To examine the relationship between the change in HbA1c and ΔAUC of postprandial blood glucose or the change in HOMA-R at week 12, correlation analysis was performed. There was significantly correlation between each parameter value of the change in HbA1c and ΔAUC of postprandial blood glucose ($P < 0.05$) or the change in HOMA-R ($P < 0.01$) (Fig. 5g).

Safety

Canagliflozin 100 mg once daily for 12 weeks was well tolerated in Japanese patients with T2DM, and none of the patients discontinued because of adverse events (AEs). Symptoms suggestive of glucose malabsorption and gastrointestinal AEs were not reported during the study period.

DISCUSSION

We found that canagliflozin 100 mg significantly increased postprandial total GLP-1 levels and transiently decreased postprandial total GIP levels in the absence of acarbose in Japanese patients with T2DM. Our findings concur with data from clinical studies with healthy subjects, where canagliflozin 300 mg decreased plasma GIP and increased plasma GLP-1 levels [9] and reduced postprandial blood glucose excursion due to delayed intestinal glucose absorption [9, 10].

Canagliflozin in combination with α-GI did not alter total GLP-1 or GIP levels, despite a significant reduction in postprandial blood glucose levels. This may be attributed to canagliflozin's lower SGLT2 selectivity over SGLT1 compared with other SGLT2 inhibitors [3], and possibly its effect on intestinal SGLT1 inhibition after oral administration. This finding was further supported by simulation studies with a physiologically based pharmacokinetic and pharmacodynamic model, in which both canagliflozin 100 mg and dapagliflozin 10 mg completely inhibited SGLT2, but not SGLT1 in the renal proximal tubule. Inhibition of SGLT1 by

dapagliflozin was less effective in the proximal intestine, whereas canagliflozin achieved 40–50% inhibition in the duodenum and jejunum within 30 min after oral administration [25]. Since intestinal SGLT1 is pivotal for the intestinal mass absorption of glucose and triggers the glucose-dependent secretion of GIP and GLP-1 [4], the inhibition of SGLT1 in the small intestine and the inhibition of SGLT2 in the renal tubules mediate canagliflozin's transient inhibition of glucose absorption.

The α-GIs delay the absorption of digested carbohydrates from the small intestine and also sustain the inhibition of the digestion of complex carbohydrates in the colon [14–16]. Furthermore, α-GIs decrease the secretion of GIP and increase the secretion of GLP-1 in the intestine [15, 16]. Indeed, our results showed that acarbose treatment significantly decreased postprandial total GIP levels and tended to increase postprandial total GLP-1 levels compared to the drug-naïve or metformin treatment before the start of canagliflozin treatment. It may be postulated that canagliflozin, after having exerted sufficient effect on α-GI, showed insufficient synergism with acarbose. On the other hand, canagliflozin added on to acarbose significantly reduced postprandial blood glucose levels. This may be attributed to the dual blocking of glucose absorption in the upper intestinal tract via SGLT1 inhibition by canagliflozin and inhibition of a portion of glucose by α-GI. Thus, we consider that the dual blocking of glucose absorption has no impact on the incretin response to the subsequent meal.

In contrast, the mechanism of canagliflozin-mediated increase in GLP-1 differs from the GLP-1 secretion mechanism by metformin. This was evident from the significant increase in GLP-1 at all time points during the MTT ($P < 0.01$), for canagliflozin added on to metformin. In patients with T2DM, metformin reportedly induces GLP-1 secretion [17–19] indirectly through an increase in bile acids in the intestine [18] or directly by an AMP-activated protein kinase (AMPK)-dependent effect [19], without delayed intestinal glucose absorption.

Postprandial glucose control [26] and intensive glycemic control reduce the risk of diabetic complications [27, 28]; the prevention of postprandial total glucose and incremental glycemic excursion decreases the risk for T2DM [29]. Reductions in HbA1c and glycoalbumin in the α-GI group were greater than those in the other groups. Although the α-GI group had improved postprandial glucose control before canagliflozin administration, its baseline HOMA-R, an index of insulin resistance, was high. In addition, the change in HbA1c level at week 12 was associated with not only the change in HOMA-R but also in part ΔAUC of postprandial blood glucose. These results suggest that canagliflozin may lead to the reduction in HbA1c levels mainly via improvement of insulin resistance by renal SGLT2 inhibition but, at least in part, via lowered postprandial glucose elevations through delayed glucose absorption in the intestine. Several studies have shown that SGLT2 inhibitors improve insulin resistance and secretion in patients with T2DM [30, 31]. In this study, no change in HOMA-B levels compared with baseline were noted in any of the canagliflozin-treated groups, because baseline HOMA-B was at a normal level.

Since the postprandial GLP-1 plays a major role in the control of glucose homeostasis [32], the extent of increased postprandial GLP-1 level on the reduction in blood glucose levels was investigated. Although we tried to analyze that the relationship between the percentage change in ΔAUC and the postprandial parameters, we could not find a correlation. A recent report showed that endogenous GIP affects postprandial plasma glucose excursions and insulin secretion more than endogenous GLP-1 in healthy individuals [33]. Therefore, a further precise study is necessary to evaluate to what extent an increased GLP-1 level contributes to a decrease in blood glucose level.

Some limitations of the present study warrant mention. First, an increased postprandial GLP-1 level by canagliflozin was associated with breakfast only and not tried with other meals. A recent report showed that canagliflozin was detected at high tissue concentration in the small intestine 24 h after administration in rats, and other SGLT2 inhibitors were not detected

[34]. Second, the effect of concomitant therapy with dipeptidyl peptidase 4 (DPP-4) inhibitors, which inhibit GLP-1 degradation and prolong its effects, was not investigated, although canagliflozin has been reported to increase the plasma level of active GLP-1 in combination with DPP-4 inhibitors [35]. Third, comparative studies with other SGLT2 inhibitors are lacking. Sotagliflozin (LX4211), a dual SGLT1/SGLT2 inhibitor, reduced postprandial hyperglycemia and GIP, and increased GLP-1 levels in patents with T2DM, and these effects are thought to be induced through a transient SGLT1 inhibition in the upper intestine [36, 37]. Our study results were similar to those reported for LX4211. Whereas, empagliflozin, which has the highest SGLT2 selectivity, increased the GLP-1 level with a single, not 4-week, administration during 3 h of fasting and 5 h after mixed meal ingestion [38], probably via a mechanism distinct from that of canagliflozin. Therefore, the aforementioned durable, additive, or synergistic effects of canagliflozin alone and in combination with DPP-4 inhibitors, and comparison with other SGLT2 inhibitor-mediated incretin secretion may be future topics of discussion in the clinical setting.

CONCLUSION

Canagliflozin 100 mg has an increased postprandial GLP-1 level in the absence of acarbose, suggesting that it may upregulate GLP-1 secretion through delayed glucose absorption in the intestine. Therefore, canagliflozin with dual actions of α-GI-like effects and renal mechanism is expected to provide a beneficial effect in the treatment of patients with T2DM.

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Compliance with Ethics Guidelines. The study protocol was approved by the Naka Kinen Clinic Institutional Review Board, Ibaraki, Japan, and is registered in the University Hospital Medical Information Network Clinical Trials Registry as UMIN000018345. All procedures performed in this study involving human participants were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1964, as revised in 2013. Informed consent was obtained from all individual participants included in the study. Participants were compensated for their time.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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